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Final Report

Development of Neurophysiological Procedures for the Detection of Organic Contaminants in Water

Sidney Weinstein, Ph. D.
Curt Weinstein, M. A.

May 1978

Supported by

U. S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

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NeuroCommunication Research Laboratories, Inc.
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Summary

The purpose of this study was to determine the feasibility of conditioning rats to detect the presence of an organic contaminant in water. In order to create a device for the practical detection of organic contaminants, this system would be made valid, precise, reliable, simple to operate, relatively inexpensive to implement and maintain, and require only a brief period of time to set up and to train personnel.

Rats were conditioned to press a lever when contaminated water (C) was presented and to refrain from this action when clean water (W) was presented. The system employed to induce operant conditioning was the use of an electrical brain stimulus (EBS) to the medial forebrain bundle (MFB) of the rat's brain, i.e., a so-called "pleasure-center" of the brain, when the rat smelled and tasted C and the presentation of a noxious loud tone (90 dB) when W was presented.

W and C were randomly presented in small dippers commonly used in research with rats. When the rat's tongue made contact with the fluid, a circuit was completed which activated the lever for EBS (when C was the stimulus) or activated the lever to deliver a noxious tone if the rat (incorrectly) pressed it when W was the stimulus.

The C employed was 2, 6 dichlorophenol in water. A saturated solution was first prepared; dilutions in distilled water to 1750, 350, 175, 17.5, 8.8, and 5.8 PPB were then prepared.

Twelve rats were trained successfully to detect C in W. Detection of the basic solution (1750 PPB) occurred in 10 rats. Eight rats also successfully detected solutions of 1:500 (350 PPB), 7 rats successfully detected 1:1000 (175 PPB), 6 rats detected 17.5 PPB, 5 rats 8.8 PPB, and 1 rat 5.8 PPB. All trained rats detected some level of C.

It is clear that rats can detect low concentrations of a contaminant in water. In order to determine the lowest possible levels of detection and the ability of the rats to detect multiple contaminants, a new study should be initiated with these goals in mind.

Foreword

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences-National Research Council.

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Rats were conditioned to press a bar if they detected contaminant (2,6 dichlorophenol) in water, and to restrain if the water was not contaminated. The conditioning resulted from the application of an electrical brain stimulation (EBS) to the Medial Forebrain Bundle (MFB) of the rat's brain (positive reinforcement) if he pressed correctly, and presentation of a loud noxious noise if he pressed incorrectly. All rats were successfully conditioned to detect the contaminant with high levels of accuracy ranging from 87-100%.

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20. (continued)

All rats were initially trained to detect contaminants at 1750 PPB. Thresholds were determined by continually training rats at decreased concentrations. Several rats were successfully conditioned to detect 2,6 dichlorophenol at a concentration of 8.8 PPB, and 1 rat at 5.8 PPB.

Body of Report

Problem

The Army had a need for sensitive, on-line procedures for the detection of organic contaminants (C) in water in order to assess the effectiveness of water purification methods. Previous research at NeuroCommunication Research Laboratories, Inc. has demonstrated the effective use of rats in detecting the odor of TNT. The purpose of this research contract was to determine whether rats similarly conditioned, could detect the presence of a common organic contaminant in water, and to estimate their threshold of detection.

Background

The history of the use of animals (dogs, dolphins, pigeons, rats, etc.) by man for various purposes to supplement his armamentarium of detection, message transmission, etc. is long and well documented (See for an excellent description " The War Dogs" by Robert E. Lubow). The use of these animals not only enhanced the abilities of man using his best equipment, but clearly made possible the performance of functions that could not have been accomplished by any other means, regardless of complexity or cost of the equipment. NeuroCommunication Research Laboratories, Inc. has completed two years of research on the use of rats to detect the presence of TNT. The results have clearly demonstrated that rats can detect the presence of TNT with high levels of precision and reliability.

The method found to be most effective in training rats to detect TNT was conditioning, using, as the conditioned (or reinforcing) stimulus, electrical brain stimulation (EBS) in one of the positive reinforcement centers (so-called "pleasure center"). The site chosen for the electrode to be implanted was the medial forebrain bundle (MFB). (See Appendix for Surgical Procedures). The rats were trained (in the operant procedure) to press a bar when TNT was present and to refrain from action when non-TNT materials were present.

Methods

Surgical Preparation. The surgical procedures to create a rat who voluntarily "self-stimulates" in order to receive an EBS have been well-established at this and other laboratories over the last few decades. The Appendix provides a detailed description of these procedures.

Training Procedures. Following surgery, each rat recovers for one week in his home cage during which time he is observed. Following recovery, he is introduced into the training cage which contains a nonretractable bar which can be activated by the experimenter. In this cage the rat is "shaped" to press the bar to receive an EBS. In order to enhance the probability that the rat will press the bar frequently, the experimenter manipulated the current levels and durations, until the maximum rate of self-stimulation was obtained for each rat. The process of "shaping" is well known by Skinnerian psychologists. Briefly, the experimenter delivers an EBS when the rat first orients toward the bar, again when it approaches the bar, and finally when it presses the bar to provide its own "self-stimulation." Rats which self-administer EBS at rates above 10/minute for at least 5 minutes were considered shaped.

When the rat became a self-stimulator he was placed in the test cage which contains a light. The rat was then trained to press the bar for EBS only when the light is on. He was taught that pressing when the light is on yields an EBS, but pressing when it is off, produces a loud, high pitched (noxious) noise.

Following this stage of training, the dippers were introduced. These dippers are wrapped with a porous spongy plastic material which contains either water (W) or 2, 6 Dichlorophenol (DCP). The tips of these dippers are removable to enable frequent random interchanging of tips on the dippers to eliminate the possibility that the rat has learned to discriminate the dippers on the basis of extraneous cues. In this final stage of training the rat was taught to "ask for a trial." This procedure is employed as follows. To ensure that the rat has actually tasted (and smelled) the dipper, we employed a Drinkometer. This device employs a circuit which is completed only when the rat touches the saturated sponge with his tongue. Thus, when the light comes on, alerting the rat that the stimuli are available for tasting, the rat then placed his tongue in contact with the stimulus. This completes one of two circuits: for C a circuit is completed which provides an EBS if the rat then (correctly) presses the bar; for W, another circuit is completed which provides a noxious tone if the rat (incorrectly) presses the bar.

With these procedures, the rats were subjected to several days of training using a saturated solution of DCP. We soon learned that this concentration was too intense (the rats showed avoidance behavior), and we replaced it with the "standard" solution 1:100 (or 1750 PPB). Rats were also given solutions of 1:500 (350 PPB) and some 1:1000 (175 PPB). Following a delay of several months, the same rats were retrained, and increasingly dilute solutions were used in an attempt to determine their absolute sensory detection thresholds.

C and W trials were always randomly presented and were equal in number in all sessions.

Variations in the number of trials given during a session resulted from sudden (inexplicable) refusal of the rats to lick the dippers, breakdown of equipment, etc.

Sessions were planned to contain 30, 40, 50, etc. trials. If the rat seemed to respond with brief latencies, a decision might be made to increase the number of trials, or to run a second (or even third) session later in the day. These modifications resulted in the trials ranging from 20-112 for all rats in a given day. Session lengths ranged from 30-60 minutes.

The statistics employed were the χ^2 test, which compares the number of correct and incorrect trials.

Results

This section provides a table (Table 1) containing the rat's identification number, the concentration of DCP tested, and the percentage correct performance. Thirty trials were routinely used, unless otherwise indicated.

Table 1

Performance of Rats in Detecting Various Concentrations of DCP

Rat No.	Concentration of DCP	% Correct	Concentration of DCP	% Correct
131	1. Saturated	99		
	2. 1750 PPB (Day 1)	88		
	3. 1750 PPB (Day 2)	88		
	4. 350 PPB	85		
	5. 175 PPB	73		
	(Lost electrical cap)			
133	1. Saturated	80		
	2. 1750 PPB	88		
	(Developed seizures, sacrificed)			
135	1. Saturated (Day 1)	66	16. Five week delay 175 PPB (Day 1) Recall of procedures to respond; NS on discrimination; 30 trials	
	2. " (Day 2)	93		
	3. 1750 PPB	98		
	4. 350 PPB	95		
	(Retesting)		17. 175 PPB (Day 2)	72 - 85 trials
	5. Saturated	95	18. 175 PPB (Day 3)	90 - 40 "
	6. 1750 PPB	93	19. 17.5 PPB	98 - 40 "
	7. 350 PPB	95	20. 8.8 PPB	93 - 40 "
	8. 175 PPB	100	(Discontinued; electrical cap loose.)	
	9. First retest after 4 month delay without training. Instant recall of procedures to respond; had to relearn discrimination.			
	1750 PPB (Day 1)	(NS)		
	10. 1750 PPB (Day 2)	90 - 60 trials		
	11. 350 PPB	95 "		
	12. 175 PPB (Day 1)	97 - "		
	13. 175 PPB (Day 2)	94 - 50 "		
	14. 17.5 PPB (Day 1)	90 - 60 "		
	15. 17.5 PPB (Day 2)	83 - 80 "		
143	Saturated	91		
	(Lost electrical Cap - Sacrificed)			
144	1. Saturated	95	9. Saturated (Day 3)	95
	2. 1750 PPB (Day 1)	73	10. Four month delay. Immediate recall of procedure, NS on discrimination	
	3. 1750 PPB (Day 2)	87	11. 1750 PPB (Day 1)	(NS)
	4. 350 PPB (Day 1)	72	12. 1750 PPB (Day 2)	86 -50 trials
	5. 350 PPB (Day 2)	62 (NS)	13. 350 PPB (Day 1)	92 -40 trials
	6. 350 PPB (Day 3)	70	14. 350 PPB (Day 2) (Some reluctance to work)	
	(Retesting)		15. 350 PPB (Day 3) (Still reluctant)	79 - 112 trials
	7. Saturated (Day 1)	87	16. 350 PPB (Day 4)	(NS)
	8. " (Day 2)	88	Refuses to work -Lost discrimination totally	

Rat No.	Concentration of DCP	%Correct	Concentration of DCP	% Correct
147	1. 1750 PPB (Day 1)	96		
	2. " " (Day 2)	96		
	3. Four month delay before retest. Immediate recall of procedures. Forgot discrimination. Recall after 34 trials.			
	4. 1750 PPB	88 -60 trials		
	5. 350 " (Day 1)	96- 50 "	14. 17.5 PPB (Day 1)	93 -40 trials
	6. 350 " (Day 2)	100 -40 "	15. 17.5 PPB (Day 2)	96 -25 trials
	7. 175 " (Day 1)	98 -40 "	16. 8.8 "	92 -25 "
	8. 175 " (Day 2)	98 - 40 "	17. 17.5 "	87 -30 "
	9. 17.5 PPB (Day 1)	100 -40 "	18. 8.8 "	95 -20 "
	10. " " (Day 2)	80 -40 "	19. 5.8 "	84 -50 "
	11. Retest after 6 week delay		20. 5.8 " (Failed)	52 -(NS)
	12. 175 PPB (Day 1)	65 -80 "		
	13. " " (Day 2)	88 -60 "		
148	1. 1750 PPB (Day 1)	87		
	2. " " (Day 2)	95		
	3. Four months delay. Immediate recall of procedures.			
	4. 1750 PPB	66-120 trials		
	5. 350 "	83 -60 "	11. 175 PPB (Day 1)	68 - 80 trials
	6. 175 " (Day 1)	93 -60 "	12. " " (Day 2)	Equipment Failure
	7. 175 " (Day 2)	85 -60 "	13. " " (Day 3)	93 - 30 trials
	8. 175 " (Day 3)	90 -20 "	14. 17.5 PPB	77 - 30 "
	9. 17.5 PPB	90 -40 "	15. 175 "	87 - 90 "
	10. One month delay		16. 17.5 "	65 - 40 "
151	1. 1750 PPB (Day 1)	87		
	2. " " (Day 2)	78		
	3. " " (Day 3)	83		
	4. (Lost electrical cap)			
153	1. 1750 PPB (Day 1)	94	10. 175 PPB Day 2)	90 -20 trials
	2. " " (Day 2)	97	11. 17.5 "	92 -60 "
	3. Retested after 2.5 months delay		12. 8.8 "	80 -40 "
	4. 1750 PPB	80 -100 trials	13. 17.5 " (Day 1)	85 -60 "
	5. 350 "	92 -50 "	14. " " (Day 2)	88 -50 "
	6. 175 "	92 -60 "	15. 175 "	92 -25 "
	7. 17.5 "	75 -60 "	16. 17.5 "	96 -25 "
	8. Three week delay		17. 8.8 "	96 -25 "
	9. 175 PPB (Day 1)	90 -60 "	18. " "	88 -25 "
			19. 5.8 "	(NS) -20 "
154	1. 1750 PPB (Day 1)	85 -60 trials		
	2. " " (Day 2)	98 -60 "		
	3. 3 month delay - not retrainable			
155	1. 1750 PPB (Day 1)	85 -60 trials		
	2. " " (Day 2)	93 -60 "		
	3. Retrained after 3 month delay - Immediate recall of procedures. Relearned discrimination at end of first retraining session.			
	1750 PPB	74 -77 trials		
	4. 350 "	87 -60 "		
	5. 175 " (Day 1)	83 -60 "		
	6. " " (Day 2)	100 -20 "		
	7. 17.5 PPB	88 -40 "		
	8. Three week delay			
	9. 175 PPB	93 -30 "		

Rat No.	Concentration of DCP	% Correct	Concentration of DCP	% Correct
155	10. 17.5 PPB	90 -30 trials	16. 175 PPB	72
(cont.)	11. 8.8 "	60 -30 "	17. 17.5 "	92 -25 trials
	12. 17.5 "	96 -25 "	18. 8.8 PPB	80 -20 "
	13. 8.8 "	70 -40 "	19. 17.5 "	80 -50 "
	14. 17.5 " (Day 1)	83 -60 "	20. 8.8 "	70 -50 "
	15. " " (Day 2) (NS)	56 -56 "		
159	1. 1750 PPB (Day 1)	87 -100 trials		
	2. " " (Day 2)	100 -60 "		
	3. Retrained after 2 month delay. Immediate recall of procedures. Forgot discrimination.			
	4. 1750 PPB (Day 1)	(NS)		
	5. " " (Day 2)	98 -60 trials		
	6. 350 " (Day 1)	90 -40 "	12. 17.5 PPB (Day 1)	96 -50 trials
	7. " " (Day 2)	100 -20 "	13. " " (Day 2)	(NS)
	8. 175 "	90 -60 "	(Equipment malfunction)	
	9. 17.5 PPB	90 -40 "	14. 17.5 PPB (Day 3)	98 -40 "
	10, One Month delay		15. 8.8 "	85 -40 "
	11. 175 PPB	94 -50 "	16. Electrical Cap loosened	

Note: Based upon the χ^2 test, the levels of statistical probability of the indicated % correct column exceed $p < .01$ except where noted as (NS). (NS) indicates failure to achieve the .01 probability or less and is therefore considered not statistically significant. It can be seen that every rat on which we initiated training achieved a level of accuracy that was highly statistically significant.

The range of % correct responses for saturated solution was 66-98%; for 1750 PPB the accuracy range was 70-100%; for 350 PPB the range was 70-100%; for 175 PPB the range was 65-100%; for 17.5 PPB the range was 65-100%; for 8.8 PPB the range was 60-96; for 5.8 PPB there was only one rat that successfully detected it, and his level was 84% for the first and 52% for the second test. No true threshold could be obtained due to the diminished number of rats that survived the long delays between initial surgery and the final test. Nevertheless, it is apparent that for the low concentrations the ability of most rats was not seriously challenged; indeed even at 8.8 PPB, the poorest rat on average was 75% accurate, and the average of the others ranged: 85, 88, 93 and 94 accurate.

Table 2 summarizes the data. The entries in the columns represent the ranked mean percentage correct performance that each rat tested achieved at each concentration. Since we learned after testing the first five rats that the saturated solution was inhibiting their performance probably due to its noxious quality, all subsequent testing was begun on 1750 PPB. We eliminated from these means the scores on 1750 PPB of the five rats that were retested after a 2.5 to 4 month hiatus. Except for this mean (89.2) the five mean scores for 1750 to 8.8 PPB show declining accuracy of performance from 91.4 to 82.5%. The lowest concentration shows a mean percentage accuracy of only 57.2%, resulting from the ability of only 1 rat to perform well (84%) and 4 rats to fail to achieve more than chance levels.

The question was also raised concerning whether the rats could retain the training after some delays. The delays (during which the rats were merely housed) ranged from 2 to 4 months (mean = 2.8 months). All 7 rats involved in the delay retained the procedures and could immediately respond. One rat (153) responded without any loss of discriminatory ability (2.5 month delay); two rats (147, 155) relearned the discrimination in one session (3 and 4 month delay); four rats (135, 144, 148, 159) required only two sessions to relearn the discrimination (4, 4, 4, and 2 month delays respectively).

Table 2

Mean Performance of Each Rat Tested at Various Concentrations

No. of Rats	Sat.	1750	350	175	17.5	8.8	5.8
1	99	96	98	98	95	94	84
2	91	96	95	92	90	93	52
3	91	96	95	92	88	88	50
4	85	95	92	91	87	85	50
5	80	94	83	86	86	70	50
6		92	87	87	85	65	
7		91	85	73			
8		89	78				
9		88					
10		88					
11		80					
M	89.2	91.4	89.1	88.6	88.5	82.5	57.2

Discussion

The results support the hypothesis that it is feasible to condition rats to detect an organic contaminant in water. The rats studied all demonstrated remarkable ease in their ability to detect DCP, in concentrations as low as 8.8 PPB.

Since it was highly exploratory, this experiment did not attempt definitively to determine the absolute threshold of detection of DCP. The effort was maximally made to answer the question: can rats detect DCP in water? Once the question was answered affirmatively, efforts were made to find absolute thresholds. These data, therefore cannot bear upon questions of variation in response among rats, variation in trials per concentration level, lack of dose response relationships, day-to-day variation in response, or effects of these variables on sensitivity of the test procedure.

This experiment, in conformity with our previous research on TNT detection, indicates that maintenance of rats with chronically implanted brain electrodes poses few problems. Infrequently, the site becomes infected; this problem is usually solved either with topical application or systemic injection of antibiotics. Other, infrequent, problems encountered are: seizures, which may not impair the rat's sensory abilities, or the physical loss of the electrical cap. On the whole, using our latest methods, rats so prepared tend to survive as long and as well as unoperated littermates.

Since only distilled water was employed in this study conclusions based upon data cannot be drawn concerning their performance with chlorine-treated water. However, in our judgment, since it was the DCP to which the rats attended, the likelihood is great that the same procedures could profitably be employed in chlorine-treated water.

The question of whether the rats could adequately perform outside the training environment is simple to deal with. The rats live in very restricted environments: home cages and test cages. The outer milieu for both these cages should produce no detectable effect upon their sensory performance.

The only questions yet to be answered, following the conclusion of this study are the range of the lowest levels rats are capable of detecting, and the degree of generality of their detection ability, i. e., can they detect multiple contaminants?

Appendix
Surgical Preparation of Rats

The following routine procedures were employed on all rats.

1. Weighing animal to determine dosage of anesthetic (Equethesin)

Dosage Chart

Standard Dosages for male Albino rats.
Supplementary dosages: 10% of original

Wt (gms)	Dosage (Ml)	Wt (gms)	Dosage (Ml)
250	.75	450	1.29
275	.81	475	1.37
300	.88	500	1.45
325	.94	525	1.53
350	1.00	550	1.61
375	1.06	575	1.63
400	1.13	600	1.77
425	1.21		

2. The anesthetic is administered I.P.
3. After 5-10 min. the operative site is shaved.
4. Mineral oil is placed in eyes to prevent dehydration.
5. Antiseptic is applied to shaved surface.
6. Rat is placed in Kopf Stereotaxic Device.
7. Incision is made about 3.0 cm long in A-P direction (See Figure 1.) just lateral to midline to avoid the saggital sinus.
8. The underlying periosteal connective tissue is scraped back to the sides exposing the skull bone. Scraping minimizes bone bleeding.
9. The sutures, the "seams" connecting the skull bones form "landmarks" to enable the location of the brain coordinates. Bregma (labled B on Fig. 1) and Lambda (labled L on Fig. 1) and the line connecting them (i.e., Midline) are the landmarks used.

The Stereotaxic device has ear bars inserted into the ear canals and a tooth bar, over which the upper teeth are placed. These hold the animal still, and enable electrode positioning. By the use of vernier scales and 3-dimensional moving units one can designate the tip of the electrode to be positioned accurately to 0.1 mm in all three dimensions. The dimensions are: Anterior-Posterior, Lateral (i.e., Right-left), and Depth.

The three coordinates are measured for Bregma and Lambda and the position of the rat is modified as needed until two of the three coordinates are identical for both points.

The depth coordinate for the medial forebrain bundle (MFB) is 8.7 mm beneath the surface. This figure is therefore subtracted from the skull surface coordinate. This point is marked and a hole burred through the skull. A needle is used to penetrate the dura mater, and bone wax used to seal the hole. Two holes are additionally drilled at the base of an equilateral triangle of which the original burr hole is the apex.

They are filled with bone wax and small screws are inserted into the holes. These screws serve to anchor the electrode when liquid cranioplastic cement is placed over them and the electrode base.

Implantation of electrode. The stereotaxic arm holding the electrode is re-adjusted to be positioned above the skull burr hole at the original coordinates arrived at to locate the original site. The electrode is then lowered into the brain until the depth reading is equal to the coordinate calculated as within the MFB. The skull site is cleaned and dried and powdered acrylic is mixed with liquid monomer (50-50) and applied directly to the skull, covering the base of the electrode. The electrical lead from the electrode is attached to a receptacle to enable us to plug in the stimulator. (See Figures 2, 3). Figures 4, 5, 6 show views of the rat's head before and after the acrylic is mounded over the receptacle to form the electrical cap. In about 10 minutes the acrylic is dried and the receptacle is released from the stereotaxic device and more acrylic is applied to reinforce the electrical cap. The skin is then sutured together around the receptacle base which has been packed with antibacterial ointment to prevent infection. This ointment is also topically applied over the sutures. The rat is then replaced on clean bedding in a recovery cage where he is kept warm by a lamp until he recovers. He is fed on wet mash and observed for a week. Typically, the postoperative period is uneventful and the rat is able to be conditioned in about 7-10 days postoperative.

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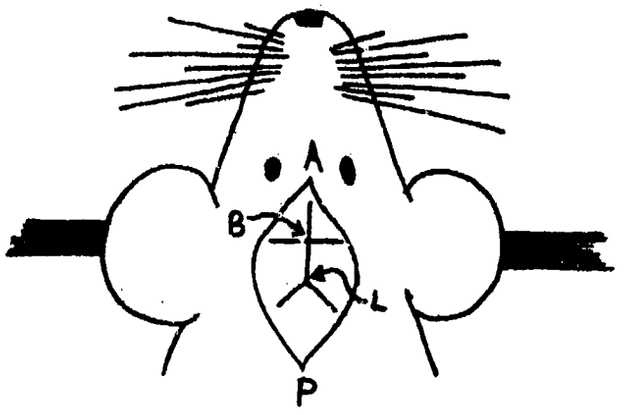
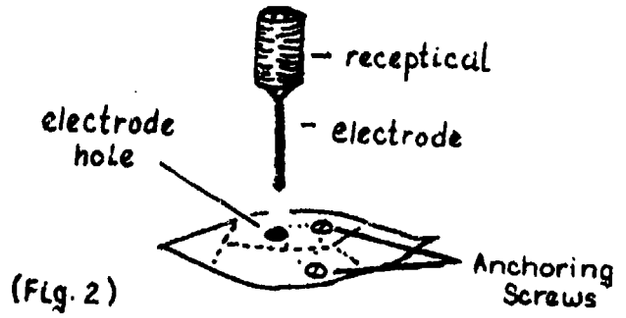


(Fig. 1)



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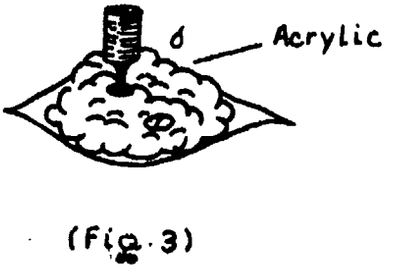

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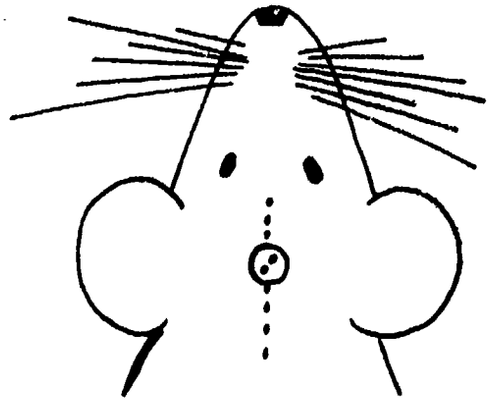
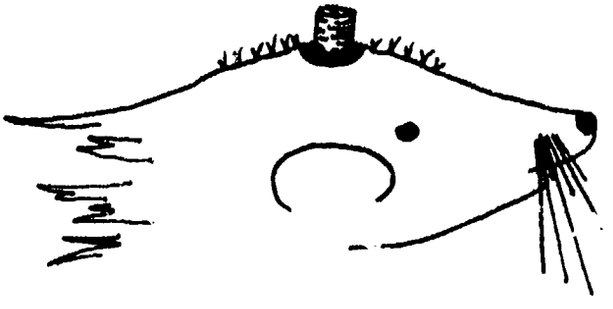


(Fig. 6)

(Fig. 4)



(Fig. 5)